



Data Article

Data on the interaction of hyperaccumulating plants with nanoscale metals Zn and Cd



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ABSTRACT

The article describes: growth phenotypes of the four plant species (*Noccaea caerulescens*, *Thlaspi perfoliatum*, *Arabidopsis halleri*, *Arabidopsis thaliana*) before and after the treatment with ionic and nanoscale Zn and Cd (Fig. 1); the method of synthesis and characterization of ZnS QDs and CdS QDs (Fig. 2); the genetic characterization (performed with molecular markers) of the four plant species, their relative genealogical relation (Fig. 3); a conceptual workflow designed to detect the amount of ionic Zn and Cd in the original solution/suspension used for the treatment (Fig. 4); the determination of Zn and Cd in the treatment soils after 30 days from supplement of ionic and nanoscale Zn and Cd (Fig. 5); the effect of the treatment on root elongation (Fig. 6); a workflow of a novel analytical method designed to detect the ionic and nanoscale Zn and Cd in the plant tissues after digestion with three different methods (Fig. 7); a reconstruction exper-

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iments with an exsiccated powder of plant tissue spiked with the same amount of Zn in the ionic and nanoscale forms (Fig. 8); a TEM-EDX analysis on these powdered plant tissues after removal of all soluble (ionic) Zn to show the presence of Zn in a non soluble form (nanoscale) (Fig. 9); the calculation of Bioconcentration Factor (BCF) and Translocation Factor (TF) and their ratios (Table 1); all data of the “spiking” experiments (Tables 2 and 3).

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Specifications Table

Subject	Plant Science: Plant Physiology
Specific subject area	Uptake and translocation of ionic and nanoscale metals
Type of data	9 Figures and 3 Tables
How the data were acquired	Uptake, translocation and accumulation data of ionic and nanoscale Zn and Cd were acquired after extraction from plant tissues and detection by FA-AAS (AA240FS equipment, Agilent Technologies, California, USA) and by ICP-MS (Optima 7300 DV device, Perkin Elmer, Waltham, MA, United States). Visualization of uptake and distribution in the tissues performed with a scanning electron microscope ESEM FEG2500 FEI (FEI Europe, Eindhoven, Netherlands) and TEM (JEOL JEM-2200FS Transmission Electron Microscope, JEOL Ltd., Tokyo, Japan).
Data format	Raw, filtered and analysed
Description of data collection	Plants hyperaccumulator and non-hyperaccumulators were treated in growth medium up to 30 days with Zn and Cd in ionic and nanoscale forms. Tissues were used for extraction and analysis of metals content with chemical (FA-AAS) and physical methods (ESEM-EDX/TEM-EDX).
Data source location	University of Parma, Parma, Italy; CINSA, Parma, Italy
Data accessibility	All data are property of the authors. All raw data are available in a data repository. Repository name: Mendeley Data URL to data: https://data.mendeley.com/datasets/2jxc648x3j/1 Data access does not require access control for ethical reasons.
Related research article	<i>D. Imperiale, G. Lencioni, M. Marmiroli, A. Zappettini, J.C. White, N. Marmiroli, Interaction of hyperaccumulating plants with Zn and Cd nanoparticles, Sci. Total Environ.</i> (2022) 152741 https://doi.org/10.1016/j.scitotenv.2021.152741

Value of the Data

- The data set provides a picture of uptake, translocation and redistribution of ionic and nanoscale Zn and Cd within Zn and Cd hyperaccumulators as compared with non-hyperaccumulator plants.
- The data can be used in planning future experiments on the interaction of plant with ionic and nanoscale metals.
- The data are, figures and tables illustrating the data collected, how they were processed (for BCF and TF) and some conceptual workflow showing how certain problems were addressed.
- The data reported may be useful for planning further experiments on the chemical environment of Zn and Cd once they were taken up either as ions or nanoscale metals. They may also be useful to establish very sensitive methods of biomonitoring (environmental).

1. Data Description

The data included in this paper are associated with a research article entitled “Interaction of hyperaccumulating plants with Zn and Cd nanoparticles” [1]. Figs. 1–3 show the phenotypic characterization of plants, of nanoscale Zn and Cd used, and the genealogical reconstruction of a tree with species related to those studied: *Noccaea caerulescens*, *Thlaspi perfoliatum*, *Arabidopsis halleri*, *Arabidopsis thaliana*.

Figs. 4–7 show the determination of metals Zn and Cd in treatment solutions, in soils, the effect of treatment on root lengths and a workflow to determine the metal distribution after differential extraction (canonical, mild acid and water) procedures on exsiccated and powdered

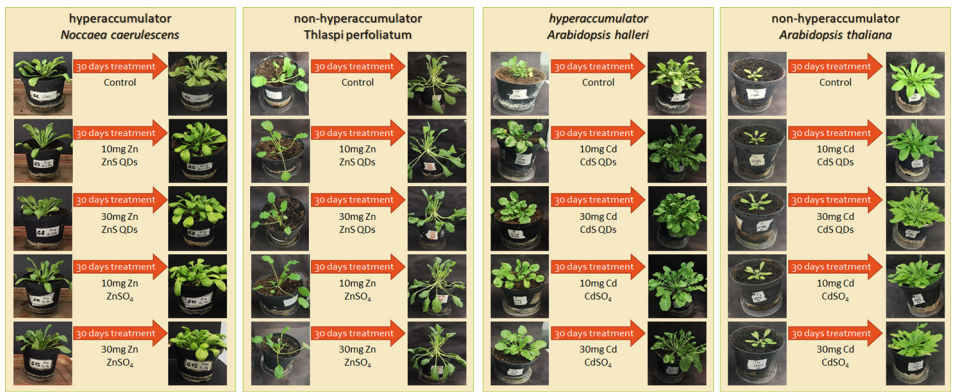


Fig. 1. Experimental design. Hyperaccumulator *N. caerulescens* and non-hyperaccumulator *T. perfoliatum* plants before and after 30 days of treatment with 10 and 30 mg of Zn administered as ZnS QDs and ZnSO₄ (above). Hyperaccumulator *A. halleri* and non-hyperaccumulator *A. thaliana* plants before and after 30 days of treatment with 10 and 30 mg of Cd administered in the nano-form of CdS QDs and in the form of CdSO₄ (below). The treatments with Zn, either ionic or nanoscale did not produce significant differences between *N. caerulescens* and *T. perfoliatum*. But the treatment with Cd evidenced important differences between *A. halleri*, hyperaccumulator and more tolerant, and *A. thaliana*, non hyperaccumulator and more sensitive. On the extreme, treatment with CdSO₄ had a positive effect on growth of *A. halleri*.

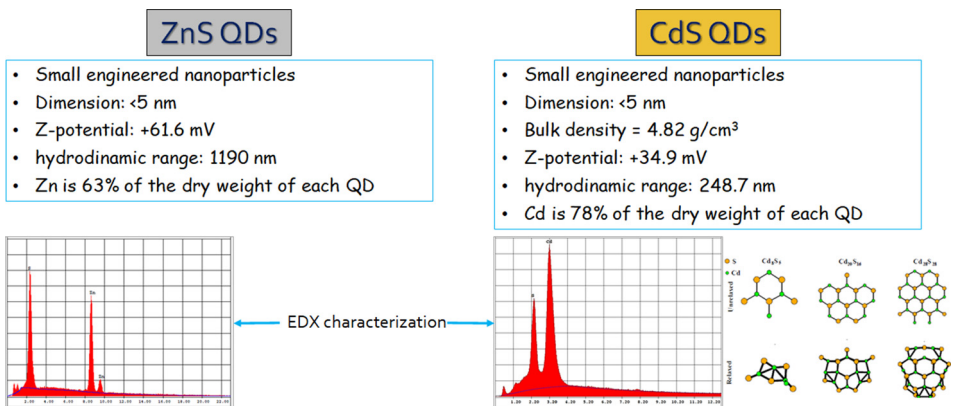


Fig. 2. Characterization of ZnS QDs and CdS QDs. After synthesis as described in the Section 2.2, the two types of quantum dots were analysed for: size/dimension, ζ -potential, hydrodynamic range and for the metal content in % of dry weight. X-Ray diffraction spectra gave for both quantum dots preparations two peaks, corresponding to the two different level of aggregation.

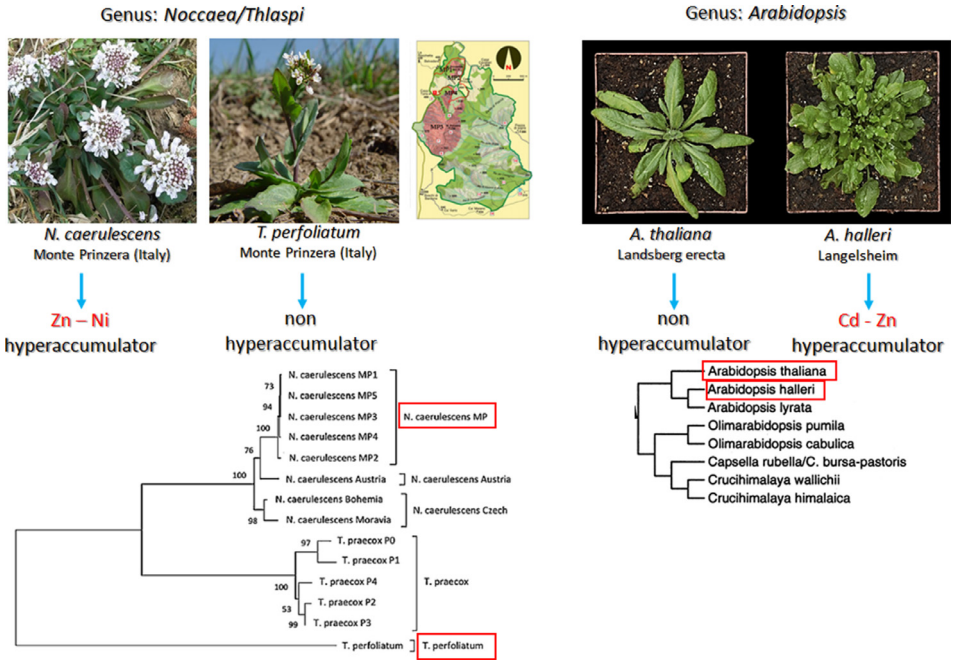


Fig. 3. Genological relation. Neighbour-Joining phylogenetic tree of several *Noccaea caerulescens* species and accessions and *Thlaspi perfoliatum*. The tree was obtained from data obtained by alignment of promoter sequences [4]. Phylogenetic tree of *Arabidopsis thaliana* and *Arabidopsis halleri* was adapted from other authors with slight modification [5].

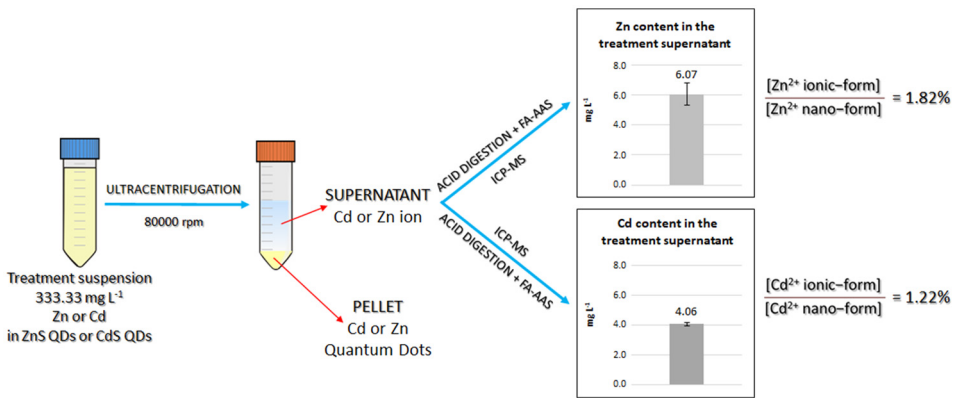


Fig. 4. Workflow of the procedure used to detect the amount of Zn and Cd (ionic-form) in the 333.33 mg L⁻¹ of Zn or Cd nanoparticle suspension. For the determination of free Zn and Cd in the 999.99 mg L⁻¹ of Zn and Cd nanoparticle suspension, the same workflow was used. The amount of free ions Zn or Cd in the supernatant did not change when the treatment suspension was made out of growth medium supplemented with 333.33 mg L⁻¹ of ZnS QDs or CdS QDs.

tissues from plants treated with ionic and nanoscale metals. Fig. 8 shows a complete reconstruction experiment where tissues powders were spiked with the same amount of Zn in ionic and nanoscale forms prior the differential extraction and analysis by ICP-MS and TEM-EDX (Fig. 9). All raw data used to analyse and prepare the figures and the tables are available from Mendeley Data repository (<https://data.mendeley.com/datasets/2jxc648x3j/1>).

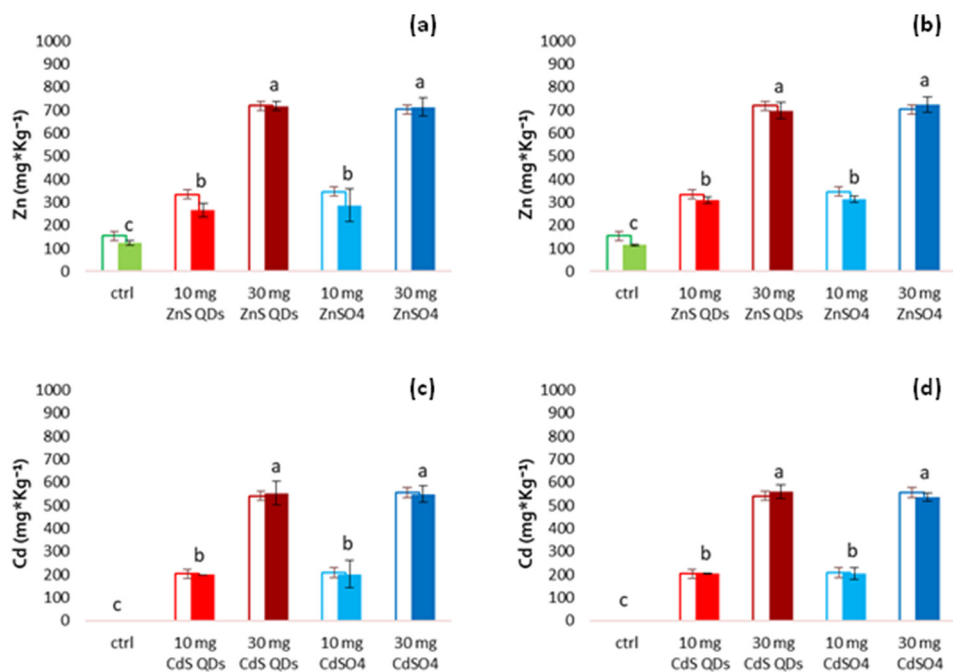


Fig. 5. Metals in soils. Zn and Cd concentration (mg kg⁻¹) in the soil of *N. caerulescens* (a), *T. perfoliatum* (b), *A. halleri* (c) and *A. thaliana* (d) measured at the beginning of the treatment (empty bars) and 30 days after the beginning of the treatment (full color bars). Different letters indicate significant differences between means in each graph at $P < 0.05$ (ANOVA and Tukey HSD post-hoc test). There were significant differences in the uptake of Zn from medium (both as ion or nanoscale) between *N. caerulescens* and *T. perfoliatum*: higher in the hyperaccumulator; smaller but significant differences were found for Cd.

2. Experimental Design, Materials and Methods

2.1. Phenotypic analysis of plants

Hyperaccumulator species (*Nocca caerulescens* and *Arabidopsis halleri*) and non hyperaccumulators (*Thlaspi perfoliatum* and *Arabidopsis thaliana*) were grown in solid medium made of sphagnum moss peat (33 %), black peat and wood fibre (66 %). The pH of this medium was 6.05 and CE was 501.3 mS m⁻¹. The medium was homogenized, sieved to 2 mm, sterilized at 120 °C and dried in an oven at 50 °C for 72 h until reaching constant weight. All treatments on plant species were done in triplicate. Each plant was grown in 300 ml plastic pots and supplemented with 10 and 30 mg of Zn and Cd in either ionic or nanoscale form. During the 30 days of continuous observation the plants were periodically checked and Fig. 1 reports the observation after 30 days.

2.2. Synthesis and characterization of ZnS QDs and CdS QDs

CdS QDs were prepared using dimethylformamide (DMF) as solvent: 10 ml of 10⁵ M thiourea were added in a 250 ml volume recipient to 100 ml to DMF. This solution was then warmed up at 70 °C when 10 ml of 10⁵ M solution of Cadmium Acetate were added, after then the temperature was raised at 90 °C. The reaction was arrested after 5 minutes, the resulting solid phase washed with distilled water and collected with centrifugation.

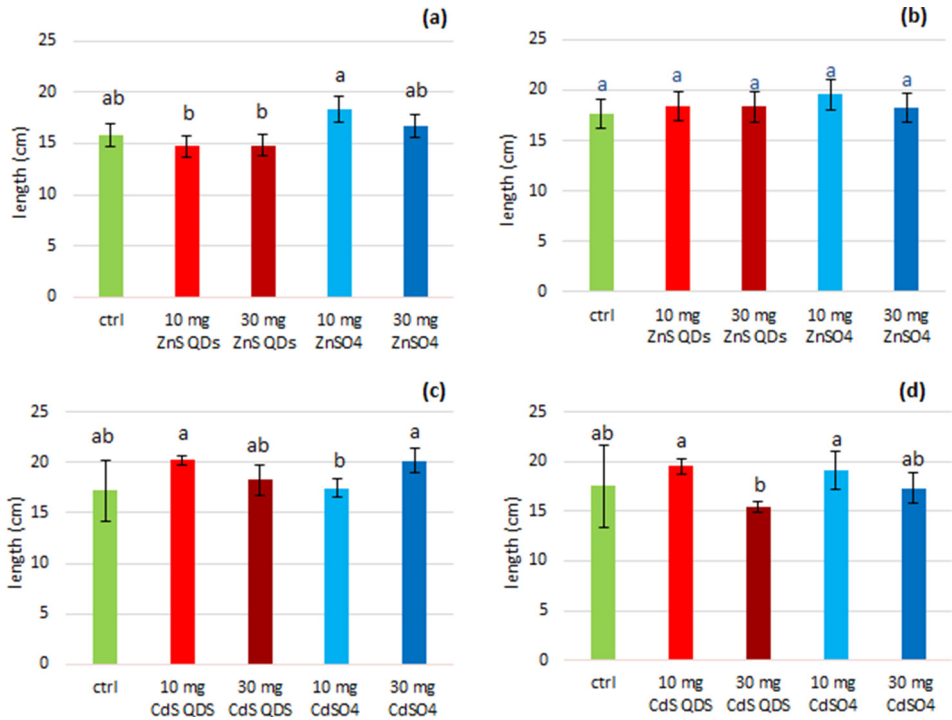


Fig. 6. Root length of *N. caerulescens* (a) and *T. perfoliatum* (b) after 30 days of treatment with Zn and *A. halleri* (c) and *A. thaliana* (d) after 30 days of treatment with Cd. Different letters indicate significant differences between means in each graph at $P < 0.05$ (ANOVA and Tukey HSD post-hoc test). The data obtained for the treatments did not show significant differences in root length after treatment between hyperaccumulators and non hyperaccumulators, whereas there were some variability within species.

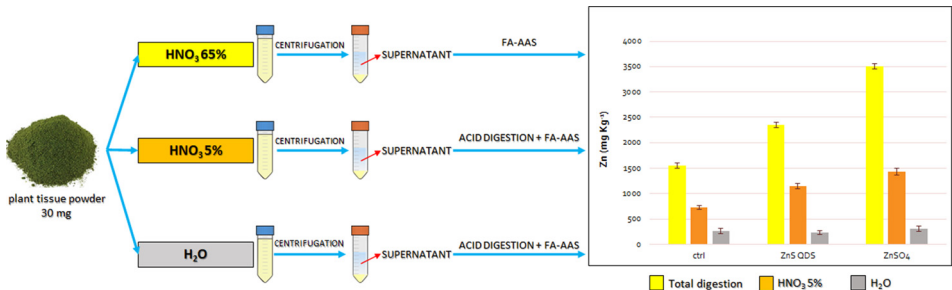


Fig. 7. Workflow of the procedure utilized to determine Zn in tissues of *N. caerulescens* digested with three different procedures, indicated by lines of different colors: yellow, orange and grey. The amount of Zn solubilized was always higher for ZnSO₄ than for ZnS QDs independently of the three methods. applied. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

The characterisation was performed on the basis of: 1- photoluminescence, performed by using 250 nm line of a laser (He-Cd) as excitation, 2- photocatalytic activity, evaluated by decomposition rate of methylene blue, 3- TEM studies on sonicated samples to determine the structural properties of single nanoparticles, 4- X-ray diffraction (XRD) performed with a thermo ARL-XTrA diffractometer, 5- Chemosensitive gas sensitive properties towards NO₂ measured by

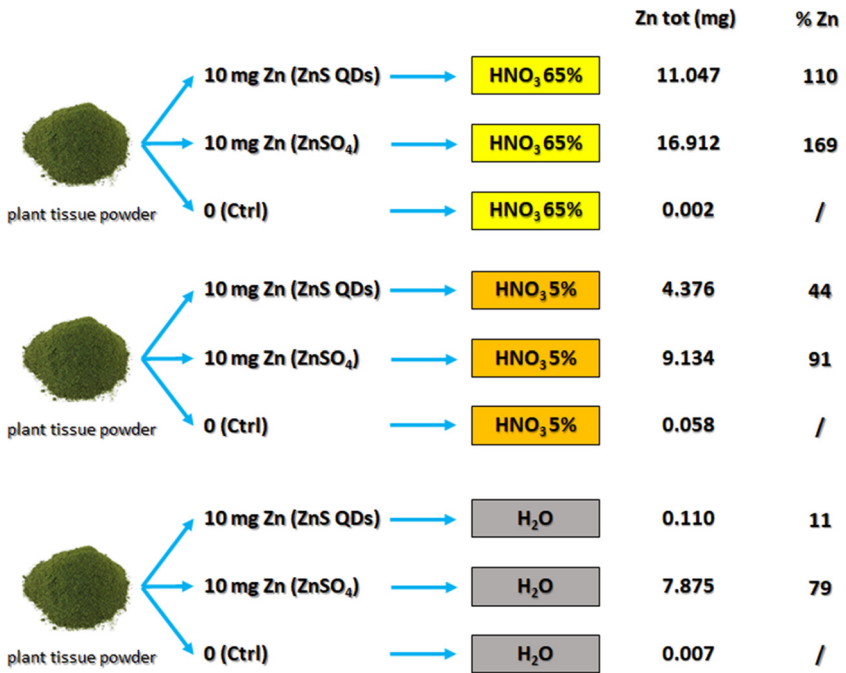


Fig. 8. Reconstruction experiment of the Zn condition in plant tissue powder after addition of ZnS QDs (10 mg of Zn) and ZnSO₄ (10 mg of Zn). The plant tissue powder was thoroughly mixed and then treated for Zn extraction according to the central column. The results obtained are those reported in Table 2. Data obtained showed that with the two mild solubilization procedures the amount of soluble Zn recovered in samples added with ZnSO₄ were 91% (HNO₃) and 79% (H₂O). For the samples “spiked” with QDs the percentages were 44% and 11% respectively. The digestion with the classical procedures (HNO₃ 65%) gave 110% and 169% respectively.

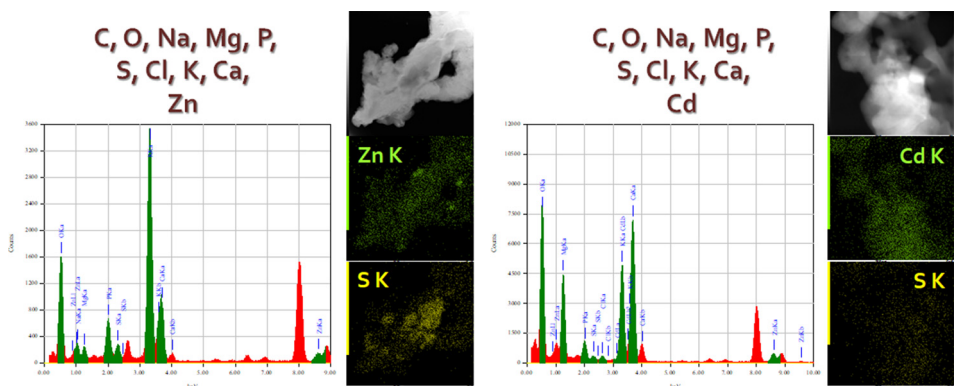


Fig. 9. TEM-EDX analysis of the plant tissue powder of *N. caeruleascens* (left) after 30 days of treatment with Zn and of *A. halleri* (right) after 30 days of treatment with Cd. On the upper right in each box, ESEM images of powder. Acceleration voltage 5 keV, pressure 60 Pa, working distance 10 mm, pinhole aperture 2.5 μm. White bar at bottom left indicates the magnification. On the left in each box: EDX spectra. Acceleration Voltage 20 keV, acquisition time 60 sec. All powders were washed out with water as in Figs. 7 and 8, dried out and analysed with TEM/EDX. The results showed the presence of significant amounts of Zn dispersed with S and K, where the original samples were spiked with ZnS QD.

a volt-amperometric procedure at constant voltage and 6- electrical properties, measuring the thermoelectric induced voltage.

Gram-scale of both CdS QDs and ZnS QDs were synthesized by optimizing a method previously reported [2,3]. X-ray diffraction (XRD) of QDs was performed using an ARL-XTra device (Thermo Fisher Scientific, MA, USA) using CuK α source in a θ - θ Bragg-Brentano geometry with an accuracy of 10^{-4} degree. For purposes of characterization, QD suspensions were prepared in MilliQ water at a concentration of 100 mg L^{-1} . To minimize aggregation suspensions were subjected to pulsed probe sonication at 40% amplitude for 2 min (Fisher Scientific Model 505 sonic dismembrator, Waltham, MA, USA). The average size (hydrodynamic diameter) and ζ -potential of each suspension was measured (Zetasizer Nano ZS, Malvern Instruments, UK) in triplicate. Information on characterization and preparation of QDs are reported in Fig. 2.

2.3. Genecological analysis of the four plant species

Plant species *Noccaea caerulescens* and *Thlaspi perfoliatum* were genetically characterized for their relatedness by studying the genetic variation in target and non-target gene sequences. Twelve different DNA fragments were amplified for each accession (sequences of primers are reported in [4]). Seven nuclear genes, promoters and two chloroplast sequences were amplified. Six out of the sequences are involved in metal homeostasis and therefore were considered target genes. A first analysis showed a certain relatedness between individuals of all the Monte Prinzera (MP) sites confirmed also by the absence of heterozygosity in the target genes. Comparing individuals from each MP sub-site with other *N. caerulescens* accessions and related species allowed the construction of a broader phylogenetic tree, with the Neighbour-Joining (NJ) method, which showed a clear separation of the different species and especially between *N. caerulescens* and *T. perfoliatum*. A similar consensus tree was obtained comparing three promoter sequences (Fig. 3). The analysis confirmed the results generated with the coding sequences. In particular the homogeneity of individual with provenience in the different MP sites chosen for sampling.

The phylogenetic tree of *A. thaliana* and *A. halleri* was adapted from other authors with slight modifications [5].

2.4. Detection Zn and Cd in different conditions and effects on growth

2.4.1. Detection in treatment solution

Characterization of treatment solutions made of Zn and Cd in ionic or nanoscale forms was performed experimentally according to the workflow showed in Fig. 4. Each treatment solution (ZnSO_4 , CdSO_4) or suspension (CdS QDs, ZnS QDs) was sonicated for 30 min at 35 kHz (Transonic T460, Elma Schmidbauer GmbH, Singen, Germany), and then digested with 5 ml of 65% HNO_3 at 165°C for 20 min with a VEP DK20 digester (Velp Scientifica S.r.l., Milano, Italy), with the addition of 1 ml of 30% H_2O_2 , eventually the solution suspension were then diluted out in 30% HNO_3 . After centrifugation at 80,000 rpm for 20 min (Optima MAX-TL, Beckman Coulter, Fullerton, CA, USA), the supernatant was analysed for the content of free metals with atomic absorption spectroscopy (FA-AAS) or ICP-MS.

2.4.2. Detection in growth medium and in plant tissues

Determination of Zn and Cd in growth medium was performed (for all the four plants species) at the beginning of the treatment and after 30 days (Fig. 5). For each sampling (medium or tissue) 50 mg were digested in 10 ml of 65% HNO_3 as described previously. Each digest was centrifuged (or filtered through a $0.45 \mu\text{m}$ filter, Sarstead, Numbrecht, Germany), and then analysed for the content of Zn or Cd as described above.

2.4.3. Effect on root elongation

Root length was measured after 30 days of treatment with Zn and Cd in ionic and nanoscale forms (Fig. 6).

2.5. Determination of Zn in tissue with three different procedures: 65% HNO₃, 5% HNO₃, H₂O

Fig. 7 shows a workflow to determine the state and concentration of Zn (mg kg⁻¹) in the supernatant of *N. caerulea* tissues after digestion with three procedures. Zn concentration in the samples after total digestion with 65% HNO₃ at 165 °C for 20 min followed by 40 min at 230 °C was reported as total Zn in control, in ZnS QDs and ZnSO₄ treatment conditions (yellow line), Zn solubilization after treatment with 5% HNO₃ (orange line) and Zn solubilization after treatment with H₂O (grey line) are also recorded. These two last treatments were also performed in the presence of small amount of 30% H₂O₂ and carried out for 90 min at room temperature.

2.6. Reconstruction experiment of the Zn condition in plant tissue powders

Powders of dried tissues of *N. caerulea* (50 mg of dry weight) were “spiked” with Zn either in ionic or nanoscale forms but with the same amount of Zn (10 mg). The “spiked” samples were subjected to three digestion procedures as described in Fig. 8 and the amount of Zn solubilized was measured by FA-AAS and ICP-MS (Table 2). In another experiment the spiked samples were digested with 5% HNO₃ and H₂O, ultracentrifuged and the Zn content was measured in the supernatant (Table 3). The precipitate was used for TEM-EDX analysis (Fig. 9).

2.7. Low-vacuum scanning electron microscope with X-ray microanalysis (ESEM/EDX)

To overcome the problems that high vacuum TEM exerts on biological samples, in this case it was used an Environmental Low-Vacuum Scanning Electron Microscope (ESEM/EDX). Sections of the biological samples (plant tissues) were cut at the microtome and fixed on the sample holder of the microscope (2 cm, stainless steel) covered with apposite adhesive carbon tape. The samples were analysed fresh.

A scanning microscope ESEM FEG 2500 FEI (FEI Europe, Eindhoven, Netherlands) operating in low-vacuum (70 Pa) with LFD (large-field detector) which allowed for an optimal secondary electron (SE) imaging. The cone PLA (Pressure Limiting Aperture) set at 500 μm improved the signal available for the Bruker XFlash[®]6 (a 30 X-ray detector equipped with a high efficiency 30 mm² SSD, Silicon Drift Detector) particularly efficient for nano-analysis and high count rate spectral imaging (Bruker Nano GmbH, Berlin, Germany). SE imaging was performed at 5 keV with a beam size of 2.5 μm and EDX analysis at 20 keV acceleration voltage and beam size of 4 μm. Working distance and scanning time were fixed empirically for each preparation. The xT Microscope Control, xT Microscope Server, and FEI User Management software were used for imaging. The Esprit 1.9 package was used for spectra acquisition and analysis. Spectra deconvolution and elemental standard-free quantification was performed using the P/B-ZAF interactive method [6].

2.8. TEM-EDX analysis of powder from tissues treated with Zn NPs

TEM analyses were made by a Field emission JEOL JEM-2200FS Transmission Electron Microscope working at 200 kV (Scherzer resolution about 0.19 nm). TEM-EDX analysis were performed on plant tissue powder of *N. caerulea* after 30 days of treatment with Zn and on *A. halleri*

after 30 days of treatment with Cd. Secondary electron imaging was performed at an acceleration voltage of 5 keV, pressure 60 Pa, working distance 10 mm, pinhole aperture 2.5 μm and EDX analysis at 20 keV, acquisition time 60 sec (Fig. 9).

2.9. Determination of bioconcentration factor (BCF) and translocation factor (TF)

Bioconcentration factors (BCF) for aerial tissues and for roots, Bioconcentration factors Ratio (BCF_{ratio}) for aerial tissues and for roots, Translocation Factor (TF) Translocation Factor Ratio (TF_{ratio}) were calculated according to the following formula:

$$BCF_{\text{aerial part}} = \frac{\text{Conc. aerial part} \left(\frac{\text{mg}}{\text{kg}} \right)}{\text{Conc. soil} \left(\frac{\text{mg}}{\text{kg}} \right)}$$

$$BCF_{\text{ratio}} = \frac{BCF_{\text{aerial part hyperaccumulator}}}{BCF_{\text{aerial part non hyperaccumulator}}}$$

Bioconcentration factor for roots and BCF_{ratio} were calculated according to:

$$BCF_{\text{roots}} = \frac{\text{Conc. roots} \left(\frac{\text{mg}}{\text{kg}} \right)}{\text{Conc. soil} \left(\frac{\text{mg}}{\text{kg}} \right)}$$

$$BCF_{\text{ratio}} = \frac{BCF_{\text{roots hyperaccumulator}}}{BCF_{\text{roots non hyperaccumulator}}}$$

Translocation Factor (TF) was calculated according to:

$$TF = \frac{\text{Conc. aerial part} \left(\frac{\text{mg}}{\text{kg}} \right)}{\text{Conc. roots} \left(\frac{\text{mg}}{\text{kg}} \right)}$$

Translocation Factor (TF_{ratio}) was calculated according to

$$TF_{\text{ratio}} = \frac{TF_{\text{hyperaccumulator}}}{TF_{\text{non hyperaccumulator}}}$$

Results obtained are presented in Table 1.

Table 1
Bioconcentration (BCF) and translocation (TF) factors.

	BCF Aerial parts [#]	BCF Roots [*]	BCF aerial parts ratio ^{##}	BCF roots ratio ^{**}	TF ^{\$}	TF ratio ^{\$\$}
<i>N. caerulescens/T. perfoliatum</i>						
ctrl	9.3/0.095	1.52/1.67	97.8	0.9	615.6/5.7	107.5
10 mg ZnS QDs	7.2/0.145	1.47/0.75	49.6	1.9	486.6/17.8	27.3
30 mg ZnS QDs	3.1/0.098	0.58/0.53	31.6	1.1	536.3/18.4	29.1
10 mg ZnSO ₄	9.4/0.204	1.36/2.45	45.9	0.6	689.9/8.3	82.6
30 mg ZnSO ₄	3.7/0.110	0.98/1.21	33.7	0.8	378.7/9.1	41.6
<i>A. halleri/A. thaliana</i>						
ctrl	0/0	0/0	0	0	0/0	0
10 mg CdS QDs	0.340/0.135	0.122/0.140	2.51	0.87	279.6/96.5	2.9
30 mg CdS QDs	0.166/0.091	0.118/0.133	1.82	0.88	140.5/68.7	2
10 mg CdSO ₄	0.405/0.153	0.065/0.244	2.64	0.26	622.2/63.0	9.9
30 mg CdSO ₄	0.276/0.124	0.118/0.120	2.22	1	234.6/103.3	2.3

BCF aerial parts (#), BCF roots (*) and the ratios (##) between the BCF aerial in *N. caerulescens* vs *T. perfoliatum* for Zn and between *A. halleri* vs *A. thaliana* for Cd. The ratios between BCF roots for Zn and Cd is also shown (**). TF values for the four types of plants and for the five different conditions of growth (ctrl, 10 mg and 30 mg CdSO₄ and CdS QDs) are shown together with the ratios of the TFs (\$\$) between hyperaccumulator vs non-hyperaccumulator for Zn and Cd.

Table 2Differential extraction of Zn from ZnSO₄ and ZnS QDs.

Sample	Zn dose of treatment	Digestion type	Zn content (mg)	Zn % compared to the treatment dose
ctrl	0	Acid (65% HNO ₃)	0.002 ± 0.0001	/
ZnS QDs	10 mg Zn as QDs	Acid (65% HNO ₃)	11.047 ± 1.165	110 ± 11
ZnSO ₄	10 mg Zn as ionic form	Acid (65% HNO ₃)	16.912 ± 3.528	169 ± 35
ctrl	0	Mild (5% HNO ₃)	0.058 ± 0.001	/
ZnS QDs	10 mg Zn as QDs	Mild (5% HNO ₃)	4.376 ± 0.002	44 ± 0.02
ZnSO ₄	10 mg Zn as ionic form	Mild (5% HNO ₃)	9.134 ± 0.006	91 ± 0.06
ctrl	0	Mild (H ₂ O)	0.007 ± 0.001	/
ZnS QDs	10 mg Zn as QDs	Mild (H ₂ O)	0.110 ± 0.001	11 ± 0.01
ZnSO ₄	10 mg Zn as ionic form	Mild (H ₂ O)	7.875 ± 0.004	79 ± 0.04

The samples (50 mg of dried powder obtained from tissues of *N. caerulea*) were added with 10mg of total Zn from either ZnSO₄ or ZnS QDs plus a control sample. The digestion was performed with 65% HNO₃, 5% HNO₃ and H₂O as described also in Figs. 6 and 8. The samples were collected and centrifuged at high speed to sediment eventual nanoparticles. The supernatants were collected and digested with 65% HNO₃ to detect the soluble Zn present. Whereas the treatment at 65% HNO₃ liberate in solution all the Zn added (either from ZnSO₄ or ZnS QDs) the treatment with 5% HNO₃ and particularly with H₂O solubilize Zn when it was from ZnSO₄ but not in from intact ZnS QDs (about 1/7 of total ZnS QDs).

Table 3Effect of mild solubilization treatments on extraction of Zn from ZnSO₄ and ZnS QDs.

Sample	Zn dose of treatment	Digestion type	Zn content (mg)	Zn % compared to the treatment dose
ctrl	0	Mild (5% HNO ₃)	0.002 ± 0.0001	/
ZnS QDs	10 mg Zn as QDs	Mild (5% HNO ₃)	6.403 ± 1.86	64 ± 18.6
ZnSO ₄	10 mg Zn as ionic form	Mild (5% HNO ₃)	12.560 ± 2.25	125 ± 22.5
ctrl	0	Mild (H ₂ O)	0.003 ± 0.001	/
ZnS QDs	10 mg Zn as QDs	Mild (H ₂ O)	0.074 ± 0.007	0.74 ± 0.1
ZnSO ₄	10 mg Zn as ionic form	Mild (H ₂ O)	10.139 ± 0.387	100 ± 3.9

The samples (50 mg of dried powder obtained from tissues of *N. caerulea*) were supplemented with 10mg of total Zn in the form of ZnSO₄ or ZnS QDs plus a non supplemented control. The samples were collected and washed with 5% HNO₃ or H₂O, ultracentrifuged to sediment Zn nanoparticles and the supernatant directly analysed for the presence of Zn. Whereas 5% HNO₃ washed out almost all Zn from ZnSO₄ and more than 50% from ZnS QDs. The washing with H₂O liberate 100% of Zn from ZnSO₄ but only 6% from ZnS QDs.

Ethics Statements

Not applicable

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Raw data on nanoscale metals Zn and Cd content in hyperaccumulating plants (Original data) (Mendeley Data).

CRedit Author Statement

Davide Imperiale: Data curation, Formal analysis, Investigation, Writing – original draft; **Giacomo Lencioni:** Formal analysis; **Marta Marmiroli:** Formal analysis, Investigation, Writing – review & editing; **Laura Paesano:** Data curation, Writing – review & editing; **Andrea Zappettini:** Validation, Writing – review & editing; **Jason C. White:** Validation, Writing – review & editing; **Nelson Marmiroli:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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